ASCITIC FLUID ADENOSINE DEAMINASE ACTIVITY – A NON INVASIVE DIAGNOSTIC TEST FOR TUBERCULOUS ASCITES

Dissertation submitted in partial fulfillment of the requirements

for the degree of

D.M. (MEDICAL GASTROENTEROLOGY)

Branch – IV

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

AUGUST 2008

CERTIFICATE

Certified that this dissertation entitled **"ASCITIC FLUID ADENOSINE DEAMINASE ACTIVITY – A NON INVASIVE DIAGNOSTIC TEST FOR TUBERCULOUS ASCITES"** is the bonafide record work done by **Dr.G.RAMKUMAR**, during the period 2005-08, under my guidance and supervision and is submitted in partial fulfillment of the requirement for the D.M. (Branch – IV) MEDICAL GASTROENTEROLOGY of The Tamil Nadu Dr. M.G.R. Medical University, August 2008 examination.

The DEAN,
Madras Medical College,
Chennai – 3.

Prof. MOHAMMED ALI, M.D., D.M.,
Professor & HOD,
Dept. of Medical Gastroenterology,
Madras Medical College,
Chennai – 3.

Date & Seal

ACKNOWLEDGEMENT

I thank Dr. T. P. Kalaniti, M.D., Dean, Madras Medical College for permitting me to carry out this study and also for providing necessary facilities.

I thank Prof. Mohammed Ali, D.M., Head, Department of Medical Gastroenterology for his valuable guidance, suggestions and constant encouragement.

My thanks are due to Prof. P. Padmanabhan, D.M., Additional Professor, Department of Medical Gastroenterology for his counselling and constructive suggestions.

I sincerely thank Prof. Srikumari Damodaram, M.Ch., Head, Department of Surgical Gastroenterology for her support and help in carrying out the study.

I express my gratitude to Dr. P. Ganesh, Dr. K. Narayanasamy, Dr. K. Premkumar and Dr. Caroline Selvi for their support, interest and enthusiasm in completion of this study.

I thank my colleagues, Dr. Rema Krishnakumar, Dr. P. Mahadevan, Dr. Antony Joe, Dr. Antoine Berty, Dr. Karthikeyan and Dr. Gokul for their help and assistance in successfully completing this study.

I extend my warm gratitude to V. Selvanarayanan, Ph.D., Professor of Entomology, Annamalai University and Master Harikumar for their help in preparing this dissertation.

I thank my family members who stood by me in successfully completing this study.

I thank all the patients who cooperated with me in carrying out this study, in spite of their illness. This work would be complete and successful, if it had contributed, even in the smallest possible way, to alleviate their suffering.

CONTENTS

INTRODUCTION

Tuberculosis has been declared a global emergency by the World Health Organization and is the most important communicable disease worldwide. The prevalence of extra-pulmonary tuberculosis seems to be rising, particularly due to increasing prevalence of acquired immunodeficiency syndrome (AIDS). In patients with extra pulmonary tuberculosis, abdomen is involved in 11% of patients. Though potentially curable, abdominal tuberculosis continues to be a major cause of morbidity and mortality in India.

In the abdomen, tuberculosis may affect the gastrointestinal tract, peritoneum, lymph nodes, and solid viscera. The disease can mimic various other gastrointestinal disorders, particularly inflammatory bowel disease, colonic malignancy, or other gastrointestinal infections. Because of the non-specific symptoms and signs, its diagnosis is often delayed.

Autopsies conducted on patients with pulmonary tuberculosis before the era of effective antitubercular drugs revealed intestinal involvement in 55-90 per cent cases, with the frequency related to the extent of pulmonary involvement. About 0.4 million people in India are co-infected with HIV and tuberculosis. Extra- pulmonary forms of tuberculosis which account for 10-15 per cent of all cases may represent up to 50 per cent of patients with AIDS. Tuberculosis of the gastrointestinal tract is the sixth most frequent form of extra-pulmonary site, after lymphatic, genitourinary, bone and joint, miliary and meningeal tuberculosis.

It is well known that confirmation of peritoneal tuberculosis is difficult and slow due to the need for histologic confirmation of caseous granulomas or bacteriologic confirmation by ascitic fluid (AF) acid-fast smears or mycobacterial

cultures. Because the results of mycobacterial cultures might take more than 4 weeks and acid-fast stained smears are disappointingly insensitive, confirmation often requires invasive procedures such as laparoscopy. Therefore a quick, noninvasive test for the diagnosis of peritoneal tuberculosis would be most helpful.

Adenosine deaminase (ADA) activity in ascitic fluid is a sensitive and specific marker for tuberculosis. Adenosine deaminase (ADA) is an enzyme widely distributed in mammalian tissues, particularly in T lymphocytes. Increased levels of ADA are found in various forms of tuberculosis making it a marker for the same. The sensitivity and sensitivity of ADA activity are 95 and 98 per cent respectively. In low protein ascites, false negative results are more frequent. In patients with HIV infection and tuberculous ascites, ADA levels may be lower. ADA is particularly useful in developing countries where more sophisticated and expensive tests such as laparoscopy may not be available.

AIM OF THE STUDY

The study was conducted with the objective of

(i) Evaluating the efficacy of ascitic fluid adenosine deaminase activity in diagnosing tuberculous ascites

(ii) The efficacy of ascitic fluid adenosine deaminase activity in differentiating tuberculous from non-tuberculous ascites

REVIEW OF LITERATURE

Abdominal Tuberculosis

Abdominal tuberculosis is defined as tuberculous infection of the abdomen including gastrointestinal tract, peritoneum, omentum, mesentery and its nodes and other solid intra abdominal organs like liver, spleen and pancreas. Mycobacterium tuberculosis is the most frequently isolated organism.

Classification of abdominal tuberculosis

1. Gastrointestinal tuberculosis

- Ulcerative
- Hypertrophic
- Sclerotic or fibrous
- Diffuse colitis

2. Peritoneal tuberculosis

- Acute tuberculous peritonitis
- Chronic peritoneal tuberculosis
    - Ascitic form
    - Encysted (loculated) form
    - Fibrous form
        - Adhesive type
        - Plastic type

3. Tuberculosis of the mesentery and its contents

- Mesenteric adenitis
- Mesenteric cysts
- Mesenteric abscess
- Bowel adhesions
- Rolled up omentum

4. Tuberculosis of the solid viscera

- Liver, biliary tract and gall bladder
- Pancreas
- Spleen

5. Miscellaneous

- Retroperitoneal lymph node tuberculosis

(1 & 2)

Peritoneal tuberculosis

Epidemiology

Peritoneal tuberculosis constitutes 4 to 10 per cent of all patients with extra pulmonary tuberculosis. It is estimated to occur in 0.1 to 3.5 per cent of patients with pulmonary tuberculosis [3, 4, 5, 6]

Pathogenesis

(a) Activation of long standing latent foci of tuberculosis infection of the peritoneum.

(b) Haematogenous spread of bacilli from an active pulmonary lesion

(c) Contiguous spread of infection from an intestinal lesion or fallopian tube – infrequent mechanism

(d) Very rarely, as a complication of peritoneal dialysis [7]

Clinical presentation

Acute Tuberculous Peritonitis

Tuberculous peritonitis has an onset closely resembling acute abdomen and such patients may often be subjected to emergency surgery. In this setting, when the abdomen is opened, straw colored fluid may be present and tubercles may be found scattered over the peritoneum and greater omentum. Sometimes, in addition to acute abdominal symptoms, ascites may be clinically demonstrable making the diagnosis of peritonitis reasonably evident [1]

Chronic Tuberculous Peritonitis

Most often, persons between 25 and 45 years of age are affected and a slight female preponderance has been observed [8]. Abdominal distension and abdominal pain are the most common presenting symptoms. Fever, weight loss and night sweats are often present. In about a quarter of the patients, an abdominal mass may be felt.

There are three types of chronic tuberculous peritonitis namely

1) Ascitic form

2) Encysted form

3) Fibrous form

1) Ascitic form

Ascitic form of Tuberculous Peritonitis often has an insidious onset. History of fatigue, weight loss, fever, anorexia, facial pallor and abdominal distension are frequently present. Abdominal pain may be present. Sometimes, considerable abdominal discomfort, diarrhoea or constipation may be present.

On physical examination, abdomen may be distended. Congenital hydroceles may be present in male children due to the processus vaginalis becoming filled with peritoneal fluid. Umbilical herniation due to increased abdominal pressure is commonly observed [2]. The rolled up greater omentum infiltrated with tubercles may be felt as a transverse solid mass in the abdomen.

2) Encysted (loculated) form:

The clinical presentation of encysted tuberculous peritonitis resembles that of the ascitic form. Patients often present with the localized abdominal distension. Diagnosis is difficult and is often made retrospectively. A child with a suspected mesenteric cyst or a female with a suspected ovarian cyst may undergo a laparotomy and an encapsulated collection of fluid may be seen. In some patients, intestinal obstruction may develop late in the disease.

3) Fibrous form:

Wide spread adhesions may cause coils of intestine especially in the ileal region to be matted together and distended. These matted coils may act as "blind-loop" leading to the development of the steatorrhoea, mal-absorption syndrome and abdominal pain. They may present as acute or sub acute intestinal obstruction.

On physical examination, the adherent loops of intestine and the thickened mesentery may be felt as lumps in the abdomen.

Tuberculosis of mesentery and its contents:

Purulent form of tuberculous peritonitis is rare and is often secondary to tuberculous salphingitis. Pus may be present amidst the mass of adherent intestinal loops and omentum. Cold abscess, entero-cutaneous and entero-enteric fistula can develop [2].

Patients with the mesenteric lymph node tuberculosis may present with fever, night sweats and abdomen pain. Enlarged mesenteric lymph nodes may be felt as lumps in the abdomen, usually in the right iliac fossa. Uncommonly, the presentation may mimic acute appendicitis. Sometimes, calcified mesenteric lymph nodes may be evident on plain radiograph of the abdomen. Sometimes, patient may present with symptoms of sub-acute intestinal obstruction.

Clinical presentation of tuberculous Peritonitis

Feature	Frequency
Abdominal distension	65 – 100%
Abdominal pain	36 – 93%
Weight loss	37 – 87%
Ascites on physical examination	55 – 100%
Abdominal tenderness	65 – 87%
Ascitic peritonitis	92 -100%
Fibroadhesive peritonitis without ascites	0 – 8 %
Diarrhoea	9 – 27%
Anemia	48 – 68%
Positive tuberculin test	55 – 100%
Abnormal chest radiograph	37 – 63%
Associated active pulmonary tuberculosis	4 – 21%

Reference [3 – 6]

Physical signs of peritoneal tuberculosis

Most patients appear ill and mal-nourished. Fever may be present. Examination of the abdomen generally reveals diffuse abdominal distension with minimal tenderness. A palpable abdominal mass may be present. The mass is due to lymph node enlargement and rolled up omentum. The classic "doughy abdomen" is present only in 10 per cent of patients.

Physical signs at presentation in patients with peritoneal tuberculosis:

Variable	Das P Shukla [10] 1976 (n – 182)	Bhansali SK [11] 1978 (n – 310)	Singh V et al [12] 1995 (n – 145)
Anemic	56.5	29	32
Malnutrition	45.6	21.7	22
Peripheral lymphadenopathy	1.6	9.0	7.0
Abdominal distension	58.2	81.3	84.2
Ascites	43.2	80.2	82.2
Rigidity/ guarding	8.7	31.7	ND

ND – not described

Weight loss is a common complaint. Anorexia contributes to marked diminution of food intake. Patients may also present with features of mal-absorption.

Fever has been reported in 40 – 70 per cent of the patients. Menstrual abnormalities have been described in nearly one third of the female patients.

Abdominal Tuberculosis in patients with Human Immuno Deficiency Virus infection and Acquired Immuno Deficiency Syndrome :

The epidemic of Acquired Immuno Deficiency Syndrome (AIDS) has been accompanied by a resurgence of tuberculosis. The incidence of extra-pulmonary tuberculosis is about 50 per cent in patients with AIDS, whereas it is 10 to 15 percent in patients without HIV infection.

Fee et al.[13] compared the presentation of abdominal tuberculosis in 43 patients infected with and 35 patients without HIV infections. Fever, weight loss and extra abdominal lymphadenopathy were more common in patients with HIV infections, whereas ascites and jaundice were more frequent in patients without HIV infection. Intra abdominal lymphadenopathy and visceral lesions were more common in patients with HIV infections, whereas ascites and omental thickening were more frequent in patients without HIV infection. Disseminated tuberculosis was present in 93 per cent of HIV infected patients compared to 31 per cent of those without HIV infection.

Investigations :

Haematology :

There is a varying degree of anemia, leucopenia with relative lymphocytosis. The erythrocyte sedimentation rate (ESR) is increased. Raised ESR was reported in 54 – 100 per cent patient in several studies [10, 11, 12]. However, ESR was found to be near normal in many histology proven patients with abdominal tuberculosis [14].

Serum Bio-Chemistry:

Serum albumin levels tend to be depressed. Serum transaminase levels tend to be normal. Serum alkaline phosphatase may be raised. A significant rise in serum alkaline phosphatase may point to the presence of granulomatous hepatitis or a lymph node compressing the biliary tree. Thus laboratory investigations are non specific and do not contribute to the diagnosis.

Mantoux test:

Positive mantoux test has been reported in 55 to 100 per cent of patients with abdominal tuberculosis. However, in areas where tuberculosis is highly endemic, positive mantoux test neither confirms the diagnosis of tuberculous ascites nor excludes it. Thus a positive tuberculin test in an appropriate clinical setting would support the diagnosis of tuberculosis.

Imaging studies:

1) Chest Radiograph:

Associated pulmonary tuberculosis has been described in 24 to 28 per cent of patients with abdominal tuberculosis.

Evidence of associated pulmonary tuberculosis in patients with abdominal tuberculosis

Variable	Das P Shukla [10] 1976 (n – 182)	Bhansali SK [11] 1978 (n – 310)	Singh V et al [12] 1995 (n – 145)
Active pulmonary tuberculosis	15.1	10.6	16.6
Pleural effusion	6.9	5.2	4.0
Healed/ calcified pulmonary tuberculosis	5.8	14.2	3.4
Total	27.8	30.0	24.0

2) Plain X-ray abdomen (erect):

Plain radiograph of the abdomen may show calcified lymph nodes or calcified granulomas in the spleen, liver and pancreas. Other radiograph features include ascites, dilatation of terminal ileum [15]

Characteristic features of ascites in plain X-ray abdomen (erect)

1. Ground glass appearance
2. Obliteration of inferior edge of liver
3. Flank stripe sign – increased space between flank stripe and ascending colon
4. Bladder ears – minimal fluid collections around the uretero vesical junction
5. "Helmer sign" – lateral edge of liver pushed away from the anterior abdominal wall.
6. Fluid in pelvis

7. Separation of bowels

Barium studies:

It is the most useful investigation for the diagnosis of intestinal tuberculosis till recently. When there is associated luminal tuberculosis in an ascitic patient, it may help to localize the lesion. Enteroclysis followed by barium enema is the best protocol for evaluation of intestinal tuberculosis. The following features are highly suggestive of intestinal tuberculosis:

1) Increased transit time with hypersegmentation and flocculation of barium is the earliest sign.

2) Localized area of irregular thickened folds, mucosal ulceration, dilated segments and strictures.

3) Linear ulcers situated along the circumference of the wall.

4) "Fleischner's" sign or "inverted umbrella" sign – Thickened ileocaecal valve which gives a broad triangular appearance with the base towards the caecum.

5) "Sterling's" sign – Rapid transit and lack of barium retention in all inflamed segment of bowels

6) String sign – Persistent narrow stream of barium in the bowel indicating stenosis.

Suri et al classified barium meal follow through findings in intestinal tuberculosis into four groups:

Group 1: Highly suggestive of intestinal tuberculosis if one or more of the following are present:

a) Deformed ileocaecal valve with dilatation of terminal ileum

b) Contracted caecum with an abnormal ileocaecal valve and/or terminal ileum

c) Stricture of the ascending colon with shortening and involvement of ileocaecal valve

Group 2: Suggestive of intestinal tuberculosis if one of the following features is present:

a) Contracted caecum

b) Ulceration or narrowing of the terminal ileum

c) Stricture of ascending colon

d) Multiple areas of dilatation, narrowing and matting of small bowel loops

Group 3: Non- specific changes:

Features of matting, dilatation and mucosal thickening of small bowel loops

Group 4: Normal study [16].

Abdominal Ultra sonography:

Abdominal ultra sonography often reveals a mass made up of matted loops of small bowel with thickened walls, diseased omentum, mesentery and loculated ascites. However, these finding are not specific to tuberculosis.

Fine septae may be seen in the ascitic fluid. These strands usually arise from the serosa of the small bowel and are due to high fibrin content of the exudative ascitic fluid. They are considered to be diagnostic of abdominal tuberculosis [15]. But this finding may also be observed in patients with malignant ascites [18].

Loculated ascites probably represents concealed peritoneal inflammation. Fluid collection in the pelvis produces thick septae and can mimic ovarian cysts. Inter-loop ascites gives rise to characteristic “club sandwich” appearance of

alternating echogenic and echo – free layer of the bowel wall and inter-loop fluid [17]. Mesentric thickening is better detected in the presence of ascites and is often seen as the "stellate sign" of bowel loops radiating out from its root.

Abdominal computerized tomography:

Abdominal CT scan is better than ultra sonography for detecting high density ascites [15]. Retroperitoneal, peri pancreatic, porta hepatits and mesenteric / omental lymph node enlargement may be evident. Abdominal CT scan also detects caseous necrosis of lymph node which appears as low attenuating necrotic centers and thick, enhancing inflammatory rim [15]. Others CT findings include thickened bowel loops and ascites. In addition to ascites, mesenteric infiltration, omental masses, peritoneal enhancement / thickening and disorganized masses of soft tissue densities may be seen. Most of the features can be seen individually in a variety of conditions like peritoneal mesothelioma, carcinomatosis and peritonitis of any form [21]. However, when a combination of these findings is associated with lymphadenopathy and mural thickening of ileocecal region, it would favour a diagnosis of tuberculosis.

ASCITIC FLUID EXAMINATION

Macroscopic appearance

- Usually straw coloured
- Can be hemorrhagic
- Rarely chylous

Ascitic fluid is usually exudative (ascitic fluid protein level greater than 2.5 g/dl). Serum ascitic fluid albumin gradient is less than 1.1g/dl in more than 90 per cent of the patients [8, 19, 20]. Ascitic fluid white blood cell count is usually 150 to 4000

cells / mm^3 and consists of lymphocytes predominantly. For unknown reasons, neutrophilic response has been observed in the ascitic fluid in patients with tuberculous peritonitis associated with peritoneal dialysis [7]. Red blood cells may be found in the ascitic fluid. Ascitic fluid reveals AFB in less than 3 per cent of the cases. In most of the series, ascitic fluid culture for Mycobacterium tuberculosis is positive in less than 20 per cent of the patients. Singh et al [4] reported that the yield of the ascitic fluid culture was as high as 83 per cent when one litre of fluid was concentrated by centrifugation and then cultured.

Adenosine deaminase activity

Adenosine deaminase (ADA) activity in ascitic fluid is a sensitive and specific marker for tuberculosis [22, 23, 24]. Adenosine deaminase (ADA) is an enzyme widely distributed in mammalian tissues, particularly in T lymphocytes. ADA is found at the cell surface of T lymphocytes and macrophages. The increase in the levels of ADA is attributed to the maturation stage and the degree of stimulation of T lymphocytes in response to cell mediated immunity to the mycobacterial antigens [25]. T lymphocytes have ADA levels 10 to 12 times higher than B lymphocytes. ADA activity varies depending on proliferative status and maturity of cells. During lymphocyte proliferation, the enzyme activity varies inversely to the maturity state of lymphocytes Increased levels of ADA are found in various forms of tuberculosis making it a marker for the same. Though ADA is also increased in various infectious diseases like infectious mononucleosis, typhoid, viral hepatitis, initial stage of HIV, and in cases of malignant tumors, the same can be ruled out clinically.

The sensitivity and sensitivity of ADA activity are 95 and 98 per cent respectively when the cut-off valve is taken as 33 U/L [24, 23, 24]. In low protein ascites, false negative results are more frequent [23]. In patients with HIV infection and tuberculous ascites, ADA levels may be lower [23]. False positive ADA result has also been reported in patients with malignant ascites [26].

The level of interferon-γ (IFN-γ) in ascitic fluid is significantly higher in tuberculous peritonitis than in malignancy and cirrhosis. Estimation of both ADA and IFN-γ levels is simple, rapid and non-invasive with high sensitivity and specificity. However, estimation of IFN-γ is almost twice expensive than ADA test. Thus ADA is particularly useful in developing countries where more sophisticated and expensive tests such as laparoscopy may not be available.

Serodiagnosis

Conventional histopathological and microbiological methods are often inadequate for diagnosing abdominal tuberculosis. In these instances, immunodiagnostic procedures seem to have a major role to play, even if they are only moderately sensitive. However, the results of various serological techniques are variable due to uncertainty of antibody response to mycobacteria, poor reproducibility and lack of specificity [27].

Polymerase chain reaction

Studies have showed that PCR assay for Mycobacterium tuberculosis in ascitic fluid is moderately sensitive in diagnosing tuberculosis ascites. Anand et al [28] have

used polymerase chain reaction (PCR) on endoscopic biopsy specimens to diagnose intestinal tuberculosis in patients with chronic diarrhea.

Scintigraphy

Radionuclide scintigraphy detects serosal inflammation and peritonitis. Gallium 67 citrate is superior to Indium 111 labelled leucocytes for detecting the areas of abdominal diseases [29]. A positive result indicates inflammation but is not diagnostic of tuberculosis.

Colonoscopy

Few patients of tuberculous ascites have an edematous or deformed ileocecal valve. Nodules, ulcers, pseudopolyps, strictures etc. may be seen. Ulceration is the most common finding and is observed in the ilieocecal region. Colonoscopic findings are not pathognomonic. Tuberculous granulomas are found in the sub-mucosa, hence multiple and deep biopsies will increase the diagnostic yield [30, 31]. A combination of histology and culture of the biopsy material can establish the diagnosis in 80 per cent of the cases [31].

Peritoneal biopsy

Blind percutaneous peritoneal needle biopsy and open parietal peritoneal biopsy under local anesthesia are also useful procedures for confirming the diagnosis of abdominal tuberculosis [10, 11, 12, 32]. The needle biopsy of peritoneum has an added limitation that an involved part of peritoneum may not be obtained [33].

Laparoscopy

Direct inspection and biopsy of the peritoneum are perhaps the most effective method of diagnosing peritoneal tuberculosis (gold standard). Laparoscopy alone will facilitate an accurate presumptive diagnosis in 95 per cent of the patients [34.] Laparoscopic biopsy specimens may reveal AFB in 75 per cent and caseating granulomas in 90 per cent of patients [34, 35].

Characteristic laparoscopic findings:

1. Multiple yellowish white miliary nodules over the visceral and parietal peritoneum.
2. Erythematous, thickened and hyperaemic peritoneum
3. Turbid or straw coloured ascites
4. Adhesions
5. Heaped up omentum
6. Rarely 'cocoon' formation

Chances of perforation are high when patients with fibroadhesive peritoneal tuberculosis are subjected to laparoscopy. In these patients, use of open exposure of the peritoneum to introduce the laparoscope will significantly reduce the risk of perforation.

Diagnosis

The following are useful in confirming the diagnosis of tuberculous ascites:

1) Abdominal paracentesis and ascitic fluid analysis (including ADA estimation),

2) Diagnostic laparoscopy and peritoneal biopsy,

3) Needle biopsy of the peritoneum and

4) Therapeutic trial with anti-tuberculosis drugs.

Diagnostic criteria for tuberculous ascites :

1. Histopathological evidence of caseating granulomas / acid fast bacilli.
2. Presence of Mycobacterium tuberculosis in sputum / tissue / ascitic fluid.
3. Clinical / radiological / operative evidence of proven tuberculosis elsewhere with good therapeutic response.
4. Good therapeutic response to anti-tuberculosis chemotherapy [44].

MATERIALS AND METHODS

The centre of study was Department of Medical Gastroenterology, in Govt. General Hospital, Madras Medical College, Chennai

Study Design	:	Prospective Diagnostic study
Venue	:	Government General Hospital, Chennai
Duration	:	24 months
Collaborating Departments	:	Department of Surgical Gastroenterology, GGH. Institute of Pathology, Madras Medical College, Chennai

About sixty patients who attended our outpatient department with history of abdominal distension of recent onset, with or without swelling of legs and / or history of jaundice were selected randomly. On clinical examination, all the patients who had ascites (either presence of shifting dullness or fluid thrill) were included.

Patient selection

Inclusive criteria

1. Patients with new onset ascites with or without pedal edema

Exclusion criteria

1. Patients with congestive cardiac failure
2. Antenatal mothers

3. Comatose patients
4. Grade III or IV Hepatic encephalopathy
5. Children less than 13 years
6. Patients admitted with history of trauma
7. H/o anti tuberculosis treatment in the past six months
8. Known abdominal malignancies on treatment
9. Patients presenting with massive upper GI bleed

Protocol

1. All patients who met the above criteria were included in the study and got admitted in our department
2. The following were noted in each patient

(i) Age

(ii) Sex

(iii) Duration of abdominal distension

(iv) H/o Swelling of legs + / -

(v) H/o Jaundice

(vi) History of fever (evening rise of temperature), night sweats

(vii) H/o Loss of appetite and H/o weight loss

(viii) H/o of G. I. Bleed

(ix) H/o altered sleep rhythm

(x) H/o Bleeding tendencies (epistaxis, gum bleeding, hematuria,etc.)

(xi) H/o Abdominal pain

(xii) H/o Breathlessness on exertion

(xiii) Past H/o jaundice

(xiv) Past H/o blood transfusions (before 1996)

(xv) Past H/o tattooing

(xvi) H/o contact with tuberculosis patient

(xvii) H/o ATT intake during childhood for primary complex

3. A thorough physical examination was done in all the sixty patients. The presence of ascites was detected clinically by either shifting dullness or by demonstrating fluid thrill. Signs of chronic liver disease with portal hypertension like palmar erythema, spider nevi, gynaecomastia, large abdominal wall collaterals (caput medusa), fetor hepaticus were noted.
4. Presence of back veins (suggestive of IVC obstruction), umbilical nodule (suggestive of peritoneal carcinomatosis), neck veins (cardiac cause of ascites) were noted.
5. Examination of cardio vascular system, respiratory system and central nervous system was done.
6. Rectal examination was done for all the patients.

Investigations

The following investigations were done in all sixty patients.

1. Complete hemogram (Hb%, TC, DC, Platelets)
2. ESR and Mantoux test
3. Liver function tests including prothrombine time / INR
4. Doppler – Ultrasonogram of the abdomen
5. Ascitic fluid analysis
6. Diagnostic upper G. I. endoscopy
7. Diagnostic laparoscopic peritoneal biopsy (only in patients with SAAG < 1.1g/dl)

Ascitic fluid analysis

Abdominal paracentesis

Patient position :

1. Patients with moderate to severe ascites – supine position, with the head of the bed or examining table slightly elevated.
2. Patients with less fluid were placed in lateral decubitus position and tapped in the right of left lower quadrant.
3. Patients with small volume of fluids were tapped with ultrasound guidance.

Choice of needle entry site :

1. Left lower quadrant (two finger breadths cephalad and two finger breadths medial to the anterior superior iliac spine).
2. An appendicectomy scar or a distended cecum with gas from lactulose therapy precludes a right lower quadrant site.

Choice of needle :

1. A standard metal 1.5 inch, 22-gauge needle was used.
2. In obese patients, 3.5 inch, 22-gauge needle was used.

Metal needles do not puncture the bowel unless there is an adherent scar or severe gaseous distension.

Technique of Aspiration

1. Sterile gloves were used when paracentesis was performed.
2. Skin was disinfected with povidine iodine solution.

3. Skin and sub-cutaneous tissues were infiltrated with local anesthetic agent (2% lignocaine).
4. To prevent leakage of fluid after the needle was withdrawn, "Z" technique was used.
5. The needle was advanced slowly through the abdominal wall in 5 mm increments. Slow insertion helped to see blood if a vessel was penetrated, allowed the bowel to move away from the needle and also allowed time for the elastic peritoneum to "tent" over the end of the needle and pierced.

'Z' Technique

The skin was displaced with one gloved hand approximately 2 cm downward and then slowly inserting the paracentesis needle mounted on the syringe held in the other hand. The hand holding the syringe stabilizes the syringe and retracts its plunger simultaneously. The skin is released only after the needle has penetrated the peritoneum and fluid flows. When the needle is ultimately removed, the skin resumes its original position and seals the needle pathway.

Amount of ascitic fluid aspirated :

1. Approximately 30 ml of ascitic fluid was aspirated
2. First, 10 ml of fluid was inoculated into the culture bottle at bed side
3. Second, 5 ml – 10 ml was placed into a EDTA tube for cell count
4. Third, 10 ml was placed in a separate tube for biochemistry
5. Fourth, 5 ml was kept separately for cytological examination.

Ascitic fluid analysis

1. The gross appearance of ascitic fluid was noted
2. The following tests were done in ascitic fluid

i) Cell count

ii) Sugar

iii) Total proteins

iv) Albumin

v) LDH

vi) Adenosine deaminase

vii) Cytological examination for atypical cells and malignant cells

Serum - Ascites Albumin Gradient (SAAG)

Serum-Ascites Albumin Gradient (SAAG) was measured by subtracting value of ascitic fluid albumin from serum albumin value. It is not a ratio. If the SAAG is 1.1 g / dl or greater, the patient can be considered to have portal hypertension, with an accuracy of approximately 97%. Conversely, if the SAAG is less than 1.1 g / dl, the patient is unlikely to have portal hypertension.

All the sixty patients underwent diagnostic upper G. I. endoscopy to screen for the presence of varices and any malignancies.

Patients who had SAAG < 1.1 under went diagnostic laparoscopic peritoneal biopsy under general anaesthesia. Samples were sent for histopathological examination.

Estimation of Adenosine- deaminase activity

Methodology

Reagents:

Microxpress ADA-MTB is the reagent.

ADA-MTB comprises of

(a) L1 – ADA- MTB Reagent – Buffer Reagent

(b) L2 – ADA-MTB Reagent – Adenosine Reagent

(c) L3 – ADA-MTB Reagent – Phenol Reagent

(d) L4 – ADA-MTB Reagent – Hydrochloride Reagent

(e) S – ADA-MTB Standard – ADA standard, ready to use

Principle:

Adenosine deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue coloured indophenol complex formed is directly proportional to the amount of ADA present in the sample.

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Ammonia} + \text{Inosine}$$

$$\text{Ammonia} + \text{Phenol} + \text{Hypochlorite} \xrightarrow[\text{Medium}]{\text{Alkaline}} \text{BLUE indophenol complex}$$

Reference value

Ascitic fluid ADA	-	Normal	< 30 U/L
		Suspect	30 – 35 U/L
		Positive	> 35 U/L

Storage:

Store the ADA – MTB kit at 2 – 8° C, away from light.

Additional material required:

Test tubes, test tube stand, water bath / incubator (37°C), distilled or deionised water, variable volume pipettes, spectrophotometer with filter at 570 – 630 nm at 37°C or colorimeter with yellow or red filter, stop watch.

Reagent preparation:

Reagents L1, L2 and standard are ready to use. Adenosine Reagent (L2) may form crystals at 2-8°C. Dissolve the same by gently warming (37°C - 50°C) the reagent for some time before use. Both the phenol reagent (L3) and hypochlorite reagent (L4) needed to be diluted 1:5 with distilled water before use (1 part of reagent + 4 parts of distilled water). The phenol reagent (L3) and hypochlorite reagent are stable for at least 6 months when stored at 2-8°C in tightly closed bottles.

Specimen collection:

Specimen must be collected before starting anti-tuberculosis therapy. Either right iliac fossa or left iliac fossa (two inches superior and medial to anterior superior iliac spine) is selected for diagnostic paracentesis. The area was disinfected and ascitic fluid collected under aseptic precautions using 18 or 22 gauge needle and “Z”

technique. No special preparation of the patient is required prior to sample collection by approved techniques. It is recommended to use fresh sample specimen for testing.

ADA is reported to be stable in biological fluids for 2 days at 2-8°C, as after this, ammonia may be released in the samples even without any microbial contamination.

Test procedure

1. Bring all reagents and samples to room temperature before use.
2. Prepare the phenol reagent and hypochlorite reagent
3. Set the spectrophotometer filter at 570 – 630 nm at 37°C
4. Pipette into clean dry test tubes labeled Blank (B), Standard (S), Sample Blank (SB) and Test (T) as follows:

Addition sequence	B	S	SB	T
	(ml)	(ml)	(ml)	(ml)
Buffer reagent	0.20	0.20	-	-
Adenosine reagent	-	-	0.20	0.20
Deionised water	0.02	-	-	-
Standard	-	0.02	-	-
Sample	-	-	-	0.02

5. Mix well and incubate at 37°C for exactly 60 minutes and then add the following

Phenol reagent	1.00	1.00	1.00	1.00
Sample	-	-	0.02	-
Hypochlorite reagent	1.00	1.00	1.00	1.00

6. Mix well and incubate at 37°C for 15 minutes or at room temperature for 30 minutes.

7. Measure the absorbance of the blank (Abs. B), Standard (Abs. S), Sample Blank (Abs. SB) and Test (Abs. T) against distilled water

Calculation

$$\text{Total ADA activity in U/L} = \frac{\text{Abs T} - \text{Abs SB}}{\text{Abs S} - \text{Abs B}} \times 50$$

Linearity

The procedure is linear upto 150 U/L. If values exceed this limit, dilute the sample with deionised water and repeat the assay. Calculate the value using this appropriate dilution factor.

Limitations

1. One unit of ADA activity releases nanomoles of ammonia in the reaction in one hour at 37°C. So patients with hyperammoniemic kidney disorders can present high levels of ADA values.
2. Patients with chronic malnutrition or HIV can present low levels of ADA values
3. Higher levels of ADA are also found in leprosy, brucellosis, viral hepatitis and infectious mononucleosis.

Diagnostic Laparoscopic peritoneal biopsy

Instruments

1. Standard light source
2. Fibreoptic cable
3. Insufflator
4. Standard 5mm trocar

5. Blunt probe, suction irrigation, atraumatic graspers, laparoscopic scissors
6. Clip appliers, staplers

Anaesthesia – General anaesthesia

Position of patient

Flat

Modified lithotomy position

Port site placement

Umbilicus – for the site of veress needle insertion and ultimately as laparoscopic port, because it is the thinnest accessible portion of the anterior abdominal wall

Position of surgeon

Surgeon is placed opposite the area being exposed and work across the midline of the abdomen

Procedure

A Hasson technique is the preferred method, however the veress technique is still acceptable. A thorough examination of the upper and lower abdomen should be carried out in a systemic fashion. A 5 mm trocar can be placed in the lower abdomen. The midline suprapubic position is preferred. An instrument to move the bowels, pelvic organs and appendix around is necessary.

All adhesions should be carefully taken to expose the area of interest. This should be done in nonvascular tissue plane and bleeding should be kept to an absolute

minimum as blood may severely limit visualization and thus the ability to complete the diagnostic evaluation. A routine order of areas to be explored will ensure all areas are explored.

Diagnostic Testing

To characterize the accuracy of diagnostic tests, four terms are routinely used.

(i) Sensitivity (True-Positive) – It provides a measure of how well the test correctly identifies patients with disease

(ii) Specificity (True- Negative) – It reflects how well the test correctly identifies patients without disease

(iii) The false negative is calculated as (1 – sensitivity)

(iv) The false positive is calculated as (1 – specificity)

A perfect test would have a sensitivity of 100% and a specificity of 100% and would completely separate patients with disease from those without it.

Calculating sensitivity and specificity require selection of a cut off point for the test to separate normal from diseased subjects. As the cut- off point is moved to improved sensitivity, specificity typically falls and vice-versa.

Measures of diagnostic test accuracy

	Disease status	
Test Result	Present	Absent
Positive	True positive (TP)	False Positive (FP)
Negative	False Negative (FN)	True Negative (TN)

Identification of the patients with disease :

$$\text{Sensitivity (True positive) rate} = \frac{TP}{TP + FN}$$

$$\text{False Negative Rate} = \frac{FN}{TP + FN}$$

True positive rate = 1 – False Negative rate

Positive predictive value = TP / TP + FP

Identification of patients without disease :

$$\text{Specificity (True Negative) rate} = \frac{TN}{TN + FP}$$

$$\text{False Positive Rate} = \frac{FP}{TN + FP}$$

True positive rate = 1 – False Positive rate

Negative predictive value = TN / TN + FN

RESULTS

Total number of patients	:	60
Male	:	34 (56.67%)
Female	:	26 (43.33%)
Age	:	13 – 65 (Mean 39.95)
Hemoglobin	:	3.7 – 13.7 gm % (Mean – 9.5 gm %)
Ascitic fluid analysis		
Total Protein	:	0.4 – 6.80g/dl (mean – 3.386)
Albumin	:	0.4 – 5.60g/dl (mean – 2.056)
Sugar	:	25 – 430mg/dl (mean – 87.516)
LDH	:	48 – 2104 (mean – 423.85)
Adenosine deaminase	:	8.0 – 212.2U/L (mean - 46.638)
SAAG	:	0.1 – 2.9g/dl (mean -1.14)

The total number of patients was divided into two groups:

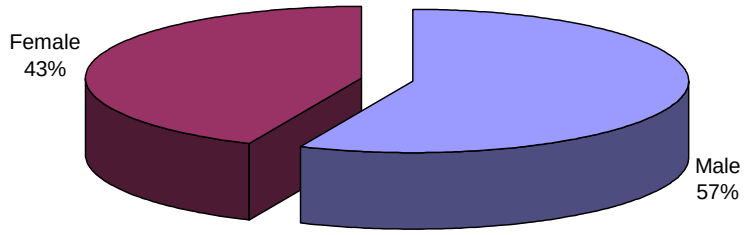
(i) SAAG < 1.1g/dl

(ii) SAAG ≥ 1.1g/dl

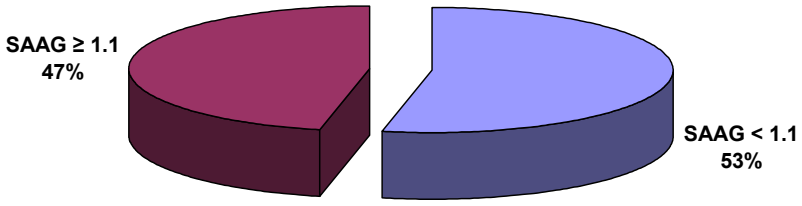
Total number of patients with SAAG < 1.1g/dl was thirty two (32) and total number of patients with SAAG ≥ 1.1g/dl was twenty eight (28).

All the thirty two (32) patients with SAAG < 1.1g/dl underwent diagnostic laparoscopic peritoneal biopsy under general anesthesia.

Gender-wise categorization of the patients



Categorization of patients based on SAAG



Etiology of patients with SAAG < 1.1 g/dl (n = 32)

Tuberculous ascites	-	27
Mixed ascites	-	2
Non Hodgkin's lymphoma	-	1
Rectal Cancer	-	1
Ovarian tumour	-	1

Etiology of patients with SAAG ≥ 1.1 g/dl (n = 28)

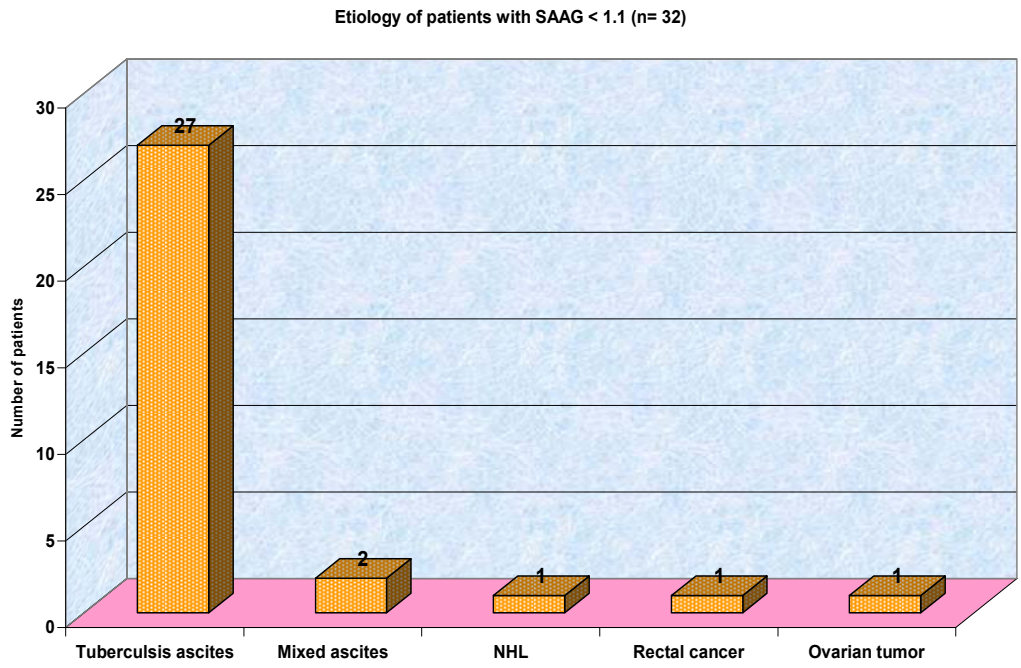
Decompensated chronic liver disease with Portal hypertension and ascites	-	25
Budd Chiari syndrome	-	1
Hepatocellular carcinoma	-	1
Cardiac ascites	-	1

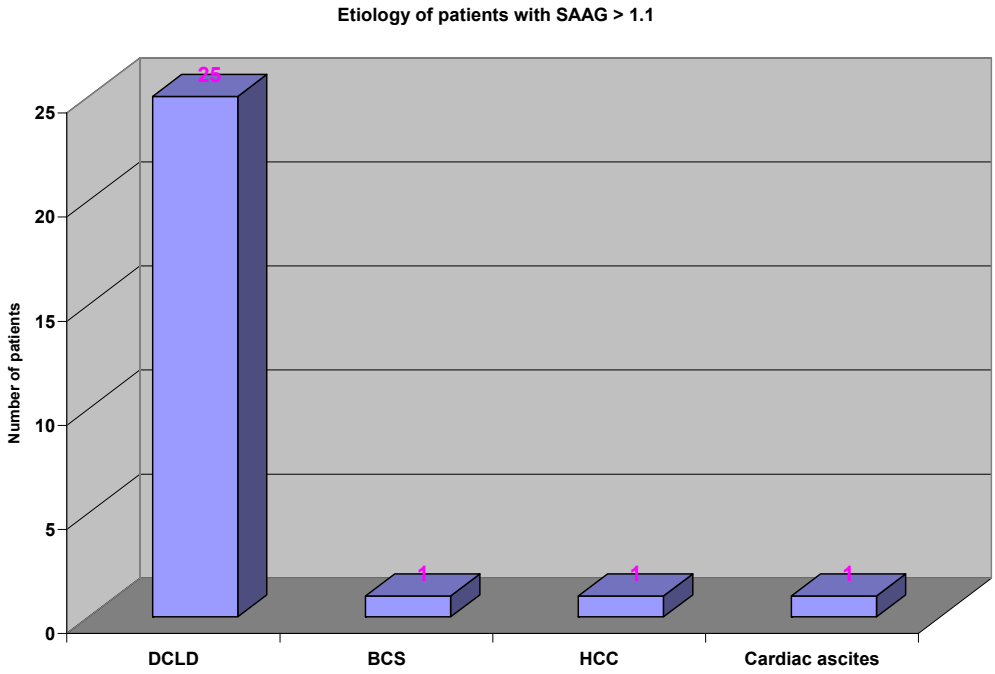
Patients were also divided into two groups based on ADA levels

1. Ascitic fluid ADA > 35 U /L
2. Ascitic fluid ADA ≤ 35 U /L

The total number of patients with adenosine deaminase activity > 35 U / L was twenty seven.

The total number of patients with adenosine deaminase activity ≤ 35 U / L was thirty three.


Etiology of patients with SAAG < 1.1 (n= 32)
Number of patients
30
25
20
15
10
5
0
27
2
1
1
1
Tuberculsis ascites
Mixed ascites
NHL
Rectal cancer
Ovarian tumor

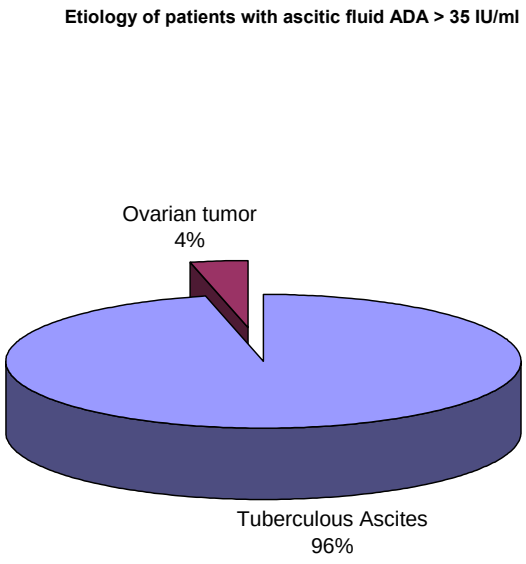

Etiology of patients with SAAG > 1.1
Number of patients
25
20
15
10
5
0
25
1
1
1
DCLD
BCS
HCC
Cardiac ascites

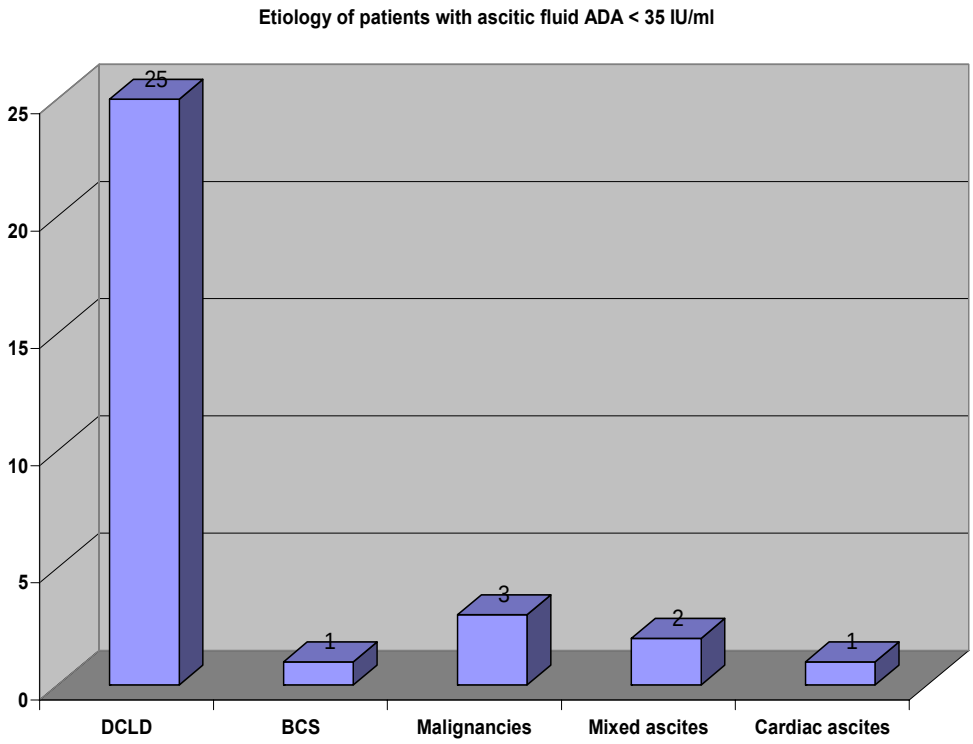
Etiology of patients with ascitic fluid ADA > 35 U /L (n = 28)

Tuberculous ascites	-	27
Ovarian tumor	-	1

Etiology of patients with ascitic fluid ADA ≤ 35 U / L (n = 32)

Decompensated CLD / PHT / ascites	-	25
Mixed Ascites	-	2
Budd Chiari syndrome	-	1
HCC	-	1
Rectal cancer	-	1
Non-Hodgkin's lymphoma	-	1
Cardiac ascites	-	1





Ascitic fluid Lactic Dehydrogenase (LDH) was also elevated two or three times the upper limit of normal value in twenty six patients of tuberculous ascites and very high values (more than 5 times the normal value) were found in all the malignancies (irrespective of the malignancies).

Decompensated chronic liver disease with portal hypertension was diagnosed by history, clinical examination, albumin globulin reversal in liver function test, presence of classical features in doppler ultrasonogram of abdomen and presence of eosophageal varices in oesophagogastroduodenoscopy.

Tuberculous ascites was confirmed by histopathological examination of peritoneal biopsy.

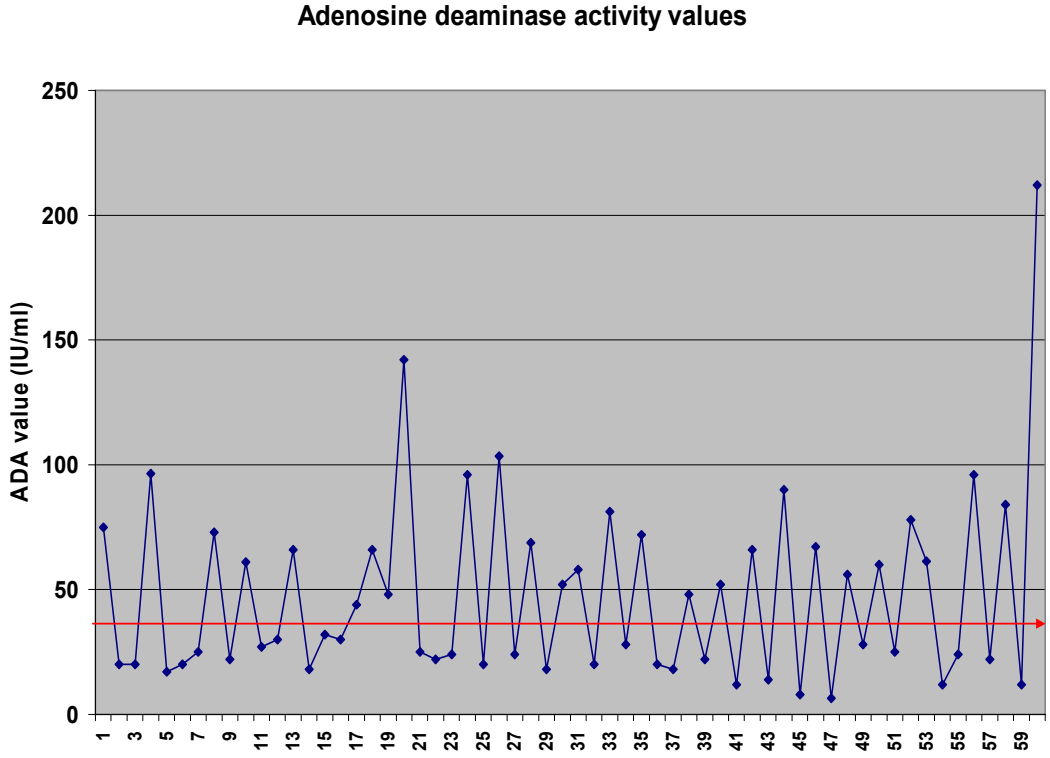
Hepatocellular carcinoma was diagnosed by radiological imaging (USG, contrast enhanced CT abdomen) and elevated serum AFP (> 400 ng/ml).

Non-Hodgkin's lymphoma was confirmed by axillary lymph node biopsy. Ovarian tumor was confirmed by CT abdomen and elevated CA-125 (> 150 ng/ml). Budd chiari syndrome was confirmed by Doppler Ultra sonogram.

In our study, the cut off value for adenosine deaminase activity was 35 U/L

	Tuberculosis	
ADA level	Present	Absent
> 35 IU / ml	n – 27	n – 1
≤ 35 IU /ml	n – 2	n – 30

1. Sensitivity = 93.1%
2. Specificity = 96.7%
3. False Negative Rate = 6.89%
4. False Positive Rate = 3.3%
5. Positive predictive value = 96.43%
6. Negative predictive value = 93.75%


Adenosine deaminase activity values
ADA value (IU/ml)
250
200
150
100
50
0
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35
37
39
41
43
45
47
49
51
53
55
57
59

DISCUSSION

About one hundred and twenty articles have been published in various national and international journals about the value of Ascitic fluid Adenosine deaminase activity either alone or in combination with interferon gamma level in discriminating tuberculosis from non-tuberculous ascites or pleural effusion. Few of them are reviewed below.

Misra SP et al did a prospective study in 49 patients (41). Adenosine deaminase was estimated in ascitic fluids of 49 patients with ascites (19 tuberculosis, 20 cirrhotic and 10 malignant). The adenosine deaminase activity in tuberculous ascitic fluid was 98.8 ± 20.1 U/L (mean ± SD), which was significantly more than noted in cirrhotic (14 ± 10.6 U/L) or malignant (14.6 ± 6.7 U/L) ascitic fluids ('P' less than 0.001 for each). At a cut off value of greater than 33 U/L, the sensitivity, specificity, positive and negative productive value, and the overall diagnostic accuracy for diagnosing tuberculous ascites were 100%, 96.6%, 95%, 100% and 98% respectively. In this study, it was concluded that estimation of adenosine deaminase activity in ascitic fluid is an easy and reliable method for diagnosing tuberculous ascites.

Bhargava DK, Gupta et al estimated ADA in six groups of patients. Simultaneous determination of ascitic fluid and serum ADA activity was evaluated as a diagnostic aid in peritoneal tuberculosis. The ascites was due to peritoneal tuberculosis (group 1), cirrhosis of the liver (group 2), cirrhosis of the liver with

spontaneous bacterial peritonitis (group 3), peritoneal malignancy (group 4), Budd-Chiari syndrome (group 5) and miscellaneous conditions (group 6). In patients with peritoneal tuberculosis, the ascitic fluid and serum ADA activity was significantly higher than for the other group ('P' less than 0.001). Levels above 36 U/L in ascitic fluid and above 54 U/L in the serum suggest tuberculosis. The ascitic fluid / serum ADA ratio was also higher in patients with peritoneal tuberculosis than with other cause of ascites ('P' less than 0.001). A ratio of more than 0.984 was suggestive of tuberculosis [24].

Gupta VK, Mukherjee S et al did a randomized control study in proven ascites patients. ADA activity in serum and peritoneal fluid was studied prospectively in 24 etiologically proved cases of ascites and 10 age matched controls. Patients were divided into 3 groups according to the cause of ascites viz.,

Group 1 : Malignant ascites (11)

Group 2 : Tuberculous ascites (7)

Group 3 : Cirrhosis of liver (6)

Serum ADA values and peritoneal: serum ADA ratio did not show any consistent pattern in any group. But in patients with tubercular peritonitis ADA activity in ascitic fluid was significantly higher ($P < 0.001$) than in the other groups. An ascitic ADA level of 30 U/L had a sensitivity of 100% and specificity of 94.1% for tuberculous ascites. This study concluded that ascitic fluid ADA activity is useful for the diagnosis of tuberculous peritonitis. This method is simple and least invasive[40].

Sathar MA, Soni PN et al did a prospective diagnostic study on the role of combined ascitic fluid gamma interferon concentrations and adenosine deaminase activity in diagnosing tuberculous peritonitis. The gamma IFN concentration and ascitic fluid ADA activity were evaluated in 30 patients with tuberculous peritonitis, 21 patients with ascites due to malignant diseases and 41 patients with cirrhosis. The gamma IFN concentrations were significantly higher ($P < 0.0001$) in tuberculous peritonitis patients (mean: 6.70 U/ml) than in the malignant (mean: 3.10 U/ml) and cirrhotic (mean: 3.08 U/ml) groups. Use of a cut off value of ≥ 3.2 U/ml gave the assay a sensitivity of 93% (25 of 27), a specificity of 98% (54 of 55), positive (P+) and negative (P-) predictive values of 96% and a test accuracy of 96% [26].

The ADA activity was significantly ($P< 0.0001$) higher in the tuberculous peritonitis group (mean: 101.84 U/L) than in the control groups (cirrhosis: mean 13.49 U/L) and malignancy (mean: 19.35 U/L). A cut off value of > 30 U/L gave the ADA test a sensitivity of 93% (26 of 28), a specificity of 96% (51 of 53), a positive predictive value of 93%, a negative predictive value of 96%, and a test accuracy of 95%. There was a significant ($P < 0.0001$) correlation ($r = 0.72$) between ADA activity and gamma IFN values in patients with tuberculous peritonitis. These results show that a high concentration of gamma IFN in ascitic fluid is as valuable as the ADA activity in the diagnosis of tuberculosis peritonitis. Both are rapid non-invasive diagnosis tests for tuberculous peritonitis.

Sharma SK, Tahir M, Mohan A et al evaluated the diagnostic accuracy of ascitic fluid interferon gamma and ADA assays in the diagnosis of tuberculous ascites. Ascitic fluid from patients with proven tuberculosis (n-31) and

non-TB ascites (n-88) was analyzed for IFN- gamma and ADA levels. Areas under the receiver operative characteristic (ROC) curves (AUCs) for the two biologic markers were compared. Levels of ascitic fluid - gamma interferon, median (range) : 560 (104-1600) pg/ml vs. 4.85 (0-320) pg/ml (P <0.001), were significantly different between TB and non-TB ascites. IFN gamma and ADA assays showed equal sensitivity (0.97) and differed marginally in specificity (0.97 vs. 0.94).Differences in AUCs was not significant (0.99 vs. 0.98, p< 0.62). For differentiating tuberculous from non- tuberculous ascites, optimal cutoff points were 112 pg/ml for IFN – gamma and 37 U/L for ADA. The accuracy of ADA assay was similar to that of the IFN-gamma assay in differentiating tuberculous ascites from non tuberculous ascites. Because both material and human costs of ADA assay are far less than those of the IFN-gamma assay, the former is the most appropriate diagnostic test for analysis of peritoneal fluid in resource limited settings [25].

Hillebrand DJ, Runyon BA, Yasmineh WG et al did a retrospective study to determine the clinical utility of ascitic fluid ADA activity in diagnosing TB ascites in a U.S patient population. 368 ascitic fluid specimens from a well characterized ascitic fluid bank, including TB ascites (n-7),mixed ascites (n-10) and consecutive specimens of varied etiologies(n-351) were analyzed by ultraviolet spectrophotometry at 265nm.The overall sensitivity of the ADA determination in diagnosing TB ascites was only 58.8% and specificity was 95.4%. However, ADA was only 30% sensitive in detecting TB ascites in the setting of cirrhosis, and cirrhosis was present in 59% of the TB ascites patients in U.S population. Malignant ascites and bacterial peritonitis specimens (5.8%) yielded false positive results. In conclusion, ascitic fluid ADA has

good accuracy but poor sensitivity and imperfect specificity in U.S population in which the prevalence of tuberculosis is low and underlying cirrhosis is common[37].

Voigt MD, Kalvaria I, Trey C et al did a retrospective study of 41 patients with bacteriologically confirmed tuberculous ascites and 41 control patients matched for age and sex, with ascites of other causes (12 alcoholic cirrhosis,5 cryptogenic cirrhosis, 12 malignant disorders, 3 pancreatic ascites and 9 miscellaneous causes). The mean ADA activity was 99.8 in TB ascites and 14.8 U/L in control patients. A cut-off of 32.3 U/L had a sensitivity of 95% and specificity of 98% in distinguishing between the two groups [23].

In a subsequent prospective study of 64 patients with ascites, 11 were found to have TB ascites. Of the others, 23 had cirrhosis,17 malignant disorders, 3 pancreatitis, 5 cor pulmonale, 3 congestive cardiac failure and 3 miscellaneous causes. The mean ascitic ADA was 112.6 IU/ml in tuberculous ascites and 16.3 U/L in those with ascites of other causes. In this study ADA had a sensitivity of 100% and specificity of 96% in discriminating tuberculosis from other causes of ascites.

P.C Mathur et al did a study on 120 patients of serosal effusion, in which 50 were of pleural effusion, 50 of peritoneal effusion and 20 cases of pericardial effusion. ADA level in tuberculous pleural effusion ranged from 45 to 160 U/L with a mean level of 100 U/L while in non tuberculous group it ranged from 5 to 33 U/L with the mean of 18 U/L.ADA level in tuberculous ascites ranged from 35 to 135 U/L with a mean level of 92 U/L while in non tuberculous group it ranged from 1 to 28 U/L with the mean of 12 U/L ($p<0.001$). ADA level in tuberculous pericardial effusion ranged from 63-117 U/L with a mean level of 90 U/L, while in non tuberculous group it ranged from 1.5 to 29 U/L with a mean of 15.33 U/L [44].

The sensitivity and specificity for diagnosing tubercular effusion was 100% and 94.6% with positive and negative predictive values of 95.5% and 100% respectively in this study.

Conrado M. et al compared the ADA in 12 patients with confirmed tuberculous ascites with that of 96 patients of other etiologies. The mean adenosine deaminase activity (ADA) value in tuberculous group was 47.9+/-21.9 and in control group was 9.6+/-5 U/L (mean+/-SD) $p < 0.01$.The best sensitivity and specificity was obtained when >32 U/L was used as a cut-off value. The ascitic fluid ADA activity correlated with ascitic fluid total protein in tuberculous group ($r = 0.842$).However false negative results occurred in those patients in which ascitic fluid total protein was low.

A recent meta-analysis reviewed twelve prospective studies. Four of them met the inclusion criteria and were thus included in the meta-analysis. The inclusion criteria were:

1) Prospective studies including consecutive patients

2) ADA levels determined by Giusti method

3) Positive mycobacterial cultures

4) Positive acid-fast stain or

5) Biopsy showing granulomatous or caseous lesions [45].

They included 264 patients, of which 50 (18.9%) had peritoneal tuberculosis. ADA levels showed high sensitivity (100%) and specificity (97%) using cut-off values from 36 to 40 IU/L. The included studies were homogeneous. Optimal cut-off value for ADA was determined at 39 IU/L, and Likelihood ratios (LRs) were 26.8 and 0.038 for values above and below this cut-off.

Study	N	GS	ADA activity/ Method	Sensitivity	Specificity	Cut-off
Sathar et al	92	B+H	Spectrophotometric method	93	96	30 U/L
Voigt et al	64	B+H	Giusti method	100	96	32 U/L
Brant et al	44	B+H	Giusti method	100	92	31 U/L
Bhargava et al	87	B+H	Giusti method	100	97	36 U/L
Sathar et al	52	H+CE	Kinetic determination	96	100	30 U/L
Martinez Vasquez et al	57	B+H+CE	Giusti method	100	95	43 U/L
In our study	60	B+H	Spectrophotometric method	93	96	35 U/L

B – Biopsy; H – Histopathology; CE – Cytological examination, GS - Gold standard

In our prospective study, Adenosine deaminase was estimated in ascitic fluids of sixty patients with ascites (27 tuberculosis, 25 cirrhotic, 4 malignant, 2 mixed ascites, 1 Budd Chiari syndrome and 1 cardiac ascites). The adenosine deaminase activity in tuberculous ascitic fluid was 78.06 ± 20.1 U/L (mean ± SD), which was significantly more than noted in cirrhotic (20 ± 10.6 U/L) or malignant (14.6 ± 6.7 U/L) ascitic fluids ('P' less than 0.001 for each). At a cut off value of greater than 35 U/L, the sensitivity, specificity, positive and negative predictive value, and the overall diagnostic accuracy for diagnosing tuberculous ascites were 93.1%, 96.7%, 96.4%, 93.7% and 98% respectively. In this study, it was concluded that estimation of adenosine deaminase activity in ascitic fluid is an easy and reliable method for diagnosing tuberculous ascites.

But the two patients with mixed ascites had a low ADA activity below the cut-off value of 35 U/L. Comparing the study by Hillebrand et al, even in south Indian population the sensitivity and specificity of ascitic fluid ADA activity is low in patients with mixed ascites.

In our study one patient with ovarian malignancy had a high ascitic fluid ADA activity (false positive).The reason for this high value is not known.

In four studies included in the metaanalysis, the cut off value for adenosine deaminase (30 or 35 IU/L) was fixed by the author. Even after the meta analysis, the cut off value for ascitic fluid adenosine deaminase was determined at 39 IU/L which is almost same as fixed in our study.

CONCLUSION

In conclusion, measurement of Adenosine deaminase activity (ADA) level in ascitic fluid is a fast and accurate test for diagnosing peritoneal tuberculosis. It has enough discriminatory power to either confirm or rule out the diagnosis of peritoneal tuberculosis in most cases. The beginning of empirical treatment when a patient has a high ADA value in ascitic fluid seems to be a good approach while waiting for the results of mycobacterial cultures or biopsies. However in the presence of cirrhosis, the sensitivity and specificity of adenosine deaminase in confirming peritoneal tuberculosis is low. Ascitic fluid adenosine deaminase can be used as a diagnostic test in centers where laparoscopy is not available. It can also be used in very sick patients who are unfit for laparoscopy.

For differentiating tuberculous ascites from non-tuberculous ascites, the optimal cut off value for asctic fluid adenosine-deaminase is 35 U/ L.

Bibliography

1. Rangabashyam N. Abdominal tuberculosis. In: Morris PJ, Malt RA, Oxford Text book of Surgery. New York, 1994. p. 2484 – 92.
2. Mann CV, Russel RCG, Williams NS, Bailey and Love's short practice of surgery. London; 1995
3. Sohocky S, Tuberculous peritonitis: a review of 100 cases: Am Review Respir Dis. 1967; 95: 398 – 401
4. Singh MM, Bhargava AN, Jain KP. Tuberculous peritonitis: an evaluation of pathogenic mechanism, diagnostic procedures and therapeutic measures; NEJM 1969; 289; 1091-94.
5. Dineen P. Homan WP, Grafe WR, Tuberculosis peritonitis: 43 years experience in diagnosis and treatment. Ann surgery 1976; 184: 717 - 72
6. Hyman S, Villa F, Alvarez S, Steigmann F: The enigma of tuberculous peritonitis. Gastroenterology. 1962; 42; 1 – 6.
7. Cheng IKP, Chan PCK, Chen MIC: TB peritonitis complicating long term peritoneal dialysis; report of 15 cases and review of literature. Am J Nephrology 1989; 9;155 – 61
8. Marshal JB, Tuberculosis of the gastro intestinal tract and peritoneum: Am J Gastroenterology 1993; 88: 989-99.
9. Bhargava DK, Shrinivas, Chopra P, Nijhawar S, Dasarathy S, Kushwaha AK, Peritoneal tuberculosis: laparoscopic patterns and its diagnostic accuracy – Am J Gastroenterology 1992; 87 : 109-17
10. Das P. Shukla Hs: Clinical diagnosis of abdominal tuberculosis Br. J. Surgery 1976; 63 : 941-6

11. Bhansali SK. The challenge of abdominal tuberculosis in 310 cases: Indian J Surgery 1978 ;40:67-77
12. Singh V, Jain AK, Agarwal AK, Khanna S, Khanna AK, Gupta JP: Clinicopathological profile of abdominal tuberculosis. Br. J. clinical practice 1995; 49: 22-24.
13. Fee MJ, Oo M.M, Gabayan AE, Radin Br, Barnes PF. Abdominal tuberculosis in patients infected with HIV. Clin Infect Dis. 1995; 20: 938- 44.
14. Gilinsky NH, Marks IN, Kotter RE, Price SK, Abdominal tuberculosis; a 1 year review, S. African Med. J. 1983; 64 : 849 – 57.
15. Dentron T, Hossain J. A radiological study of abdominal tuberculosis in a Saudi population, with special reference to ultrasound and computer tomography. Clin radiology. 1993 ; 47 ; 409-14
16. Suri S Kaur H, Wig JD, Singh K. CT in abdominal tuberculosis – comparison with barium studies. Indian J radio. Imaging. 1993 ; 3 ; 237-42
17. Keder RP, Shah PP, Shivde RS, Malde HM, Sonographic findings in peritoneal tuberculosis. Clin. Radiology. 1994; 49 ; 24-29.
18. Kompels BM, Darlington LA; Ultrasonic diagnosis of tuberculous peritonitis. Br. J Radiol 1978; 57; 1018-9.
19. Marshall JB, Vogele KA, serum - ascites albumin difference in tuberculosis peritonitis. Am J Gastroenterology 1988; 85 :59 – 61
20. Runyon BA Montano AA, Akriviadis EA, Antillon MR, Iriving MA, Mc Hutchison JG, The serum – ascites albumin gradient is superior to the exudate – transudate concept in the difference diagnosis of ascites. Ann Intern Med. 1992; 117; 215 – 20.

21. Epstein BM, Mann JH, CT of abdominal tuberculosis; AJR Am J Roent 1990; 155: 1241 – 44.

22. Martinez – Vazquez JM, Ocana I, Segura Rm. ADA activity in the diagnosis of peritoneal tuberculosis. Gut 1986; 27: 1049 – 53.

23. Voigt MD, Kalvaria I, Trey C, Berman P, Lombard C, Diagnostic value of ADA in tuberculous peritonitis; Lancet 1989; 1: 751 – 54.

24. Bhargava DK, Gupta M, Dasarathy S, Nijhawan S, Kushwaha AK, ADA in peritoneal tuberculosis; diagnostic value in ascitic fluid and serum. Tubercle 1990; 71: 121 – 6

25. Sharma SK, Mohan A. Adenosine Deaminase in the diagnosis of tuberculous pleural effusion. Indian J chest Dis Allied Sci 1996; 38: 69 – 71

26. Sathar MA, Simjee AE, Coovadia YM, Soni PN, Moolasa, Insam B, et al. ascitic fluid gamma interferom concentrations and ADA activity in tuberculous peritonitis. Gut 1995; 36: 419 – 21.

27. Daniel TM, Antibody and antigen detection for the immunodiagnosis of tuberculosis: why not? what more is needed? Where do we stand today? J Infect Disease 1988;158: 678-80.

28. Anand BS, Schneider FE, El-Zaatari FA, Shawar RM, Clarridge JE, Graheam DY .Diagnosis of peritoneal tuberculosis by polymerase chain reaction on biopsy specimens. Am J Gastroenterology 1994;89:2248-49.

29. Pettengell KE, Larsen C, Garb M, Mayet FG, Simjee AE, Pirie D, Gastrointestinal tuberculosis in patients with pulmonary tuberculosis. Q J Med. 1990;74:303-8

30. Singh V, Kumar P, Kamal J, Prakash V, Vaiphei K, Singh KW, Clinicopathological profile of colonic tuberculosis. Am J Gastroenterology 1996, 91: 565-568.

31. Pettengell KE, Pirie D, Simjee AE. Colonoscopic features of early intestine tuberculosis. Report of 11 cases. South African J. of Medicine, 1991, 79: 279-80.

32. Shukla HS, Bhatia S, Das P, Gupta SC. Peritoneal biopsy for the diagnosis of Koch's abdomen. Post Graduate Med J, 1982, 58: 226-28.

33. Mehrotra MP, Mathur KS, Agarwal AN. Value of peritoneal biopsy in clinically diagnosed cases of tuberculosis. JAPI, 1966; 12: 625.

34. Wolfe JH, Behn AR, Jackson PT. Tuberculous peritonitis and role of diagnostic laparoscopy. Lancet, 1979; 1: 852-853.

35. Menziez RI, Fitz Gerald JM, Mulpeter K. Laparoscopic diagnosis of ascites. Br. Med J. 1986; 291: 473-75.

36. Chuttani HK, Sarin SK. Intestinal tuberculosis. Indian J Tubercle. 1985; 35: 117-19.

37. Hillebrand DJ, Runyon BA, Yasmineh, WG, Rynders GP. Ascitic fluid adenosine deaminase insensitivity in detecting tuberculous peritonitis in the United States. Hepatology, 1996, 24(6): 1408-12.

38. Brant CQ, Silva MR, Jr, Macedo EP, Vasconcelos C, Tamaki N, Ferraz Ml.The value of adenosine deaminase (ADA) determination in the diagnosis of tuberculosis ascites. Rev Inst Med Trop Sao Paulo. 1995. 37(5): 449-53.

39. Fernandez-Rodriguez CM, Perez-Arguelles BS, Ledo L, Garcia-Vila LM, Periera S, Rodriguez-Martinez. Ascites adenosine deaminase activity is

decreased in tuberculosis ascites with low protein content. Am J Gastrotenterol. 1991. Oct; 86(10): 1500-3.

40. Gupta VK, Mukherjee S, Dutta SK, Mukherjee P. Diagnostic evaluation of ascitic ADA in tuberculous peritonitis. JAPI 1992 June; 40(6): 387-9.
41. Dwivedi M, Misra SP, Misra V, Kumar R. Value of adenosine deaminase estimation in the diagnosis of tuberculous ascites. Am J Gastroenterol. 1990. Sep; 85(9): 1123-5.
42. Martinez-Vazquez JM, Ocana I, Ribera E, Sequra RM, Pascual C. Adenosine deaminase activity in the diagnosis of tuberculous peritonitis. Gut 1986 Sep; 27(9): 1049-53.
43. Vardareli E, Kebapci M, Saricam T, Pasaoglu O, Acikalin M. Tuberculous peritonitis of the wet ascitic type: clinical features and diagnostic value of image-guided peritoneal biopsy. Dig Liver Dis. 2004 Mar; 36(3):199-204.
44. P.C.Mathur, K.K.Tiwari, Sushma Trikha, Dharmendra Tiwari. Diagnostic value of Adenosine deaminase (ADA) activity in Tubercular serositis. Indian Journal of tuberculosis, 2006;53: 92-95
45. Arnoldo Riquelme, MD, Mario Calvo, MD,w Felipe Salech, MD, Sebastia´n Valderrama, MD, Alejandro Pattillo, MD,* Marco Arellano, MD, Marco Arrese, MD, Alejandro Soza, MD,Paola Viviani, MS,z and Luz Marı´a Letelier, MDy. Value of Adenosine Deaminase (ADA) in Ascitic Fluid for the Diagnosis of Tuberculous Peritonitis - A Meta-analysis . J Clin Gastroenterol 2006;40:705–710

APPENDIX

ASCITIC FLUID ADENOSINE DEAMINASE – A NON-INVASIVE DIAGNOSTIC TEST FOR TUBERCULOSIS ASCITES

PROFORMA

S. No.

Name : Age: Sex:

Occupation:

Address:

Contact No.:

Hospital No.:

Symptoms:

i) Abdominal distension

ii) Swelling of legs

iii) Jaundice

iv) Blood vomiting

v) Fever

vi) Loss of appetite & loss of weight

vii) Abdominal pain

viii) Altered sleep rhythm / altered sensorium

ix) Bleeding tendencies

PAST HISTORY

i) Jaundice

ii) Abdominal surgeries

iii) Blood transfusion

iv) Hepatotoxic drug intake

v) Tattooing

PERSONAL HISTORY

i) Alcohol

ii) Smoking

iii) Drug abuse

iv) Marital Status

v) Promiscuity

EXAMINATION

Signs:

Consciousness	:	Orientation	:
Fever	: Y / N	Clubbing	:
Pallor	: Y/ N	Cyanosis	:
Jaundice	: Y / N	Pedal edema	:
Lymphadenopathy	:	JVP	:
Oral cavity	:		
Signs of CLD	:		
Cutaneous bleed	:		
Ascites	:		

Hepatosplenomegaly :

Abdominal wall collaterals :

Asterixis :

INVESTIGATIONS

1. Complete Hemogram

   Hb% Platelets

   TC ESR

   DC

   Mantoux

2. Urine analysis
3. Stool examination
4. Blood sugar
5. Serum creatinine Blood urea
6. Liver function tests

T. Bilirubin	:	SAP	:
D. Bilirubin	:	T. Protein	:
ID. Bilirubin	:	Albumin	:
AST	:	Globulin	:
ALT	:	A/ G	:

7. PT / INR :
8. Alfa-feto protein :
9. X- ray chest and abdomen :
10. Ascitic fluid analysis

Cell count	:	Cytology	:
Sugar	:		

T. Protein :

Albumin :

LDH :

Adenosine deaminase

11. Diagnostic Upper GI Endoscopy

12. Ultrasound abdomen with doppler

13. Diagnostic laparoscopic peritoneal biopsy

(a) Laparoscopic findings

(b) Histopathology of biopsy specimens

ABBREVATIONS:

P – Polymorphs

L – Lymphocytes

E – Eosinophils

M – Mesothelial cells

FF – Free fluid

C – Cirrhosis

P – Portal hypertension

A – Ascites

BCS – Budd Chiari syndrome

Ov Tr – Ovarian tumour

H – Hepatomegaly

S – Splenomegaly

V – Varices

N – Normal